JACKFISH BAY: ACUTE LETHALITY AND CHLOROPHENOL BIOCONCENTRATION IN FISH EXPOSED TO A BLEACHED KRAFT MILL EFFLUENT (BKME) PLUME

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Ontario
Ministry of the Environment

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by

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INTRODUCTION

This report presents an assessment of the acute effects of the Kimberly Clark pulp mill effluent on fish caged in Jackfish Bay, Lake Superior. The study was undertaken during July, 1983 as a joint venture of the Ministry's Northwestern Region and Toxicity Unit of the Water Resources Branch. The results of this study provide a follow up to work conducted by Cherwinsky (1983), which characterized contaminants within the Kimberly Clark mixing zone during 1981.

The objectives of this study were to:

1. determine the extent of fish lethality within the pulp mill plume in Jackfish Bay.

2. determine the cause/effect relationship between the plume characteristics and field bioassay results.

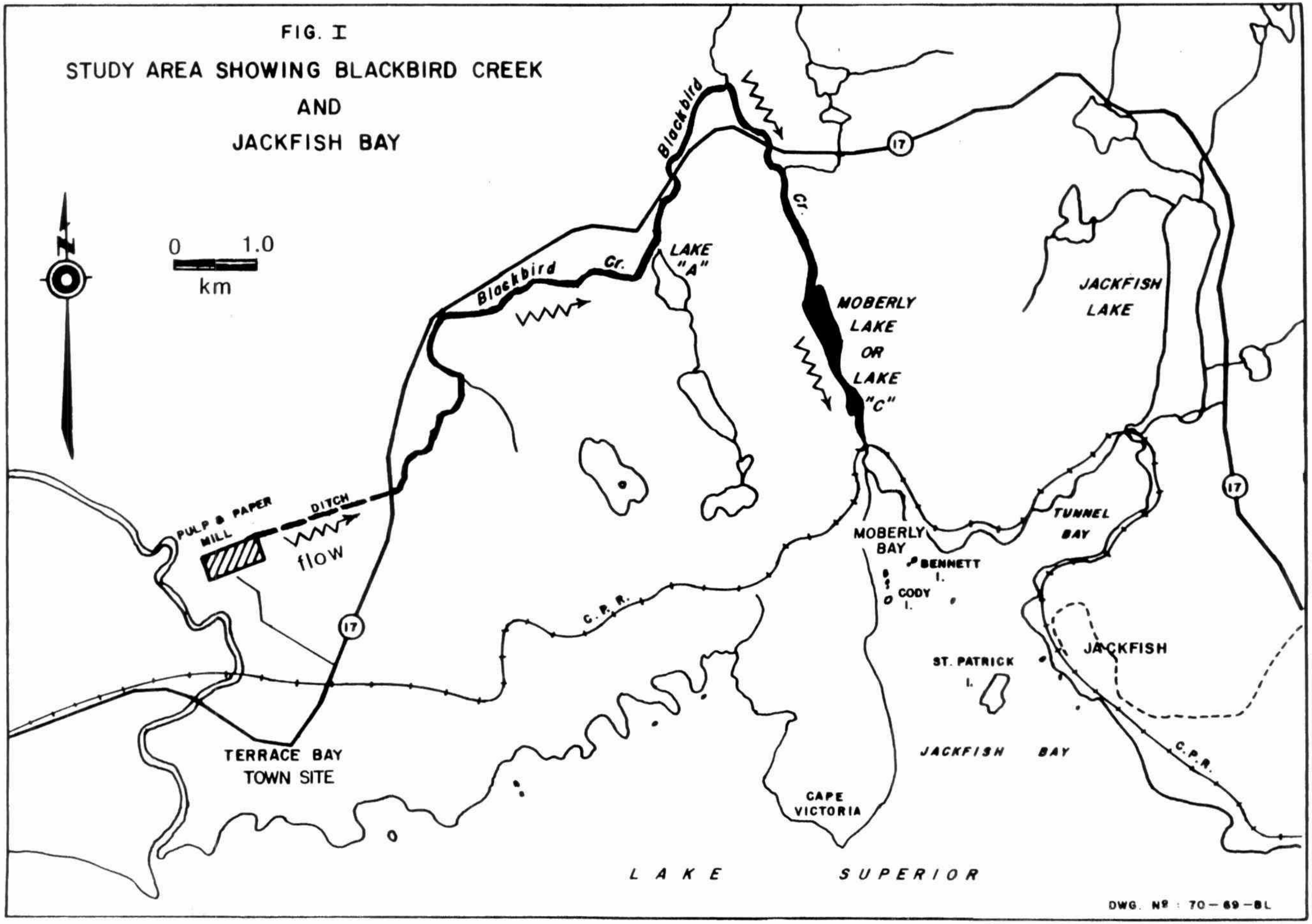
3. compare the laboratory and field bioassay results.

4. assess the usefulness of short-term fish exposures in an effluent plume to determine the bioconcentration potential of trace contaminants in fish.

Kimberly Clark of Canada operates a high grade bleach kraft mill, which is located on the north shore of Lake Superior in the township of Terrace Bay (Figure 1). At the time of the survey, it had a design production rate of 1135 tonnes of pulp per day, using a 100% softwood feedstock. An effluent volume of up to 160,000 cu.m per day was discharged from the mill, through a 15 km stretch of Blackbird Creek to Moberly Bay, the western arm of Jackfish Bay.

FIG. I

STUDY AREA SHOWING BLACKBIRD CREEK AND JACKFISH BAY



During a reconstruction program from November 1981-February 1982; the mill undertook several steps to reduce the losses of black liquor and other toxic components into the total mill effluent (TME) and to reduce water consumption. The company is committed to an in-plant toxicity reduction program which will reduce liquor and lime losses to the sewers, provide better brownstock washing and improve the quality of condensates. Furthermore, the mill proposes, by in-plant processes, to eliminate approximately 85% of the toxic components from the TME in order to meet the toxicity requirements of the Federal Pulp and Paper Effluent Regulations (Env. Can., 1971), as required by the 1982 MOE Control Order.

In 1981, eight laboratory bioassays of the Kimberly Clark effluent showed it to be acutely lethal with a 96-hour LC50* which ranged from 6-17%. The discharge to Moberly Bay was only slightly less lethal with LC50's ranging from 8-21% (N=8). A previous study has identified the accumulation of guaiacol and phenol in the sediments of the bay, while trichlorophenol levels in the surface waters have ranged from 3300 ng/L (ppt) at the creek discharge, to levels of 200 ng/L found 1.5 km out into the bay. (Cherwinsky, 1983)

Trichlorophenol levels have been reported in fish caught near pulp and paper mill discharges. Paaisvirta *et al* (1980) found pike with 13.6 $\pm$ 17.1 ng/g of 2,4,6-trichlorophenol, 5 km downstream of a pulp mill in Finland; while Bacon (1978) reported elevated 2,4,6-trichlorophenol levels in tissues of fish near a pulp mill discharge at St. Johns, N.B.

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*Footnote: a 96-hour LC50 is the lethal concentration in a bioassay at which 50% of the fish die over a 96-hour period.

The uptake of chlorophenols by fish was studied in this project because they:

i) are common to many pulp mill wastes;

ii) are present in the Kimberly Clark mill effluent (Cherwinsky, 1983);

iii) bioconcentrate in fish primarily from water rather than up the food chain (Niimi and Cho, 1983) - i.e. no feeding required during the study;

iv) can be taken up by the fish within a short period of time - i.e. in a matter of hours (Glickman *et al*, 1977);

v) are considered by the federal government as Priority Substances for future investigation and potential regulation under the Environmental Contaminants Act (Can. Test Ltd. and EVS Consultants, 1979); and

vi) are considered as deleterious to the environment by the IJC, in their recommendation that, "The pulp and paper industry should discontinue the use of trichlorophenol and pentachlorophenol as slimicides". (IJC, 1981).

Footnote: The Kimberly Clark (Terrace Bay) mill discontinued the use of phenol-based slimicides several years prior to this study.

METHODS

1. Study Outline

Hatchery-reared rainbow trout were exposed in cages at various sites in the vicinity of the Blackbird Creek discharge to Moberly Bay from July 10-14/83. Daily fish mortality observations and water chemistry sampling were undertaken at each exposure site.

A concurrent program at the Thunder Bay mobile lab involved 96-h acute bioassays on daily samples of the mill effluent and Blackbird Creek discharge.

Upon completion of the study, fish surviving the 96-hour *in situ* exposure were sacrificed and preserved for chlorophenol analyses.

2. Field Exposures

a) Test Fish

Rainbow Trout (*Salmo gairdneri*) fingerlings were supplied by Goossen's Trout Farm of Otterville, Ontario and shipped by air to Thunder Bay. Upon arrival, they were acclimated at the MOE/EPS mobile laboratory for 10 days prior to the exposure. Thunder Bay tap water was dechlorinated and delivered to the test stocks at temperatures ranging from 11-14.5^{o}C. The fish loading rate during acclimation was 0.5-0.7 L/g/day and the mean fish weight was 9.1 ± 3.4 g (N=25).

The fish were transported to Terrace Bay in an insulated fibreglass-lined container of laboratory water and were supplied a constant flow of pure oxygen during the 3-hour journey. At Terrace Bay, batches of 50 fish were placed into individual food-grade polyethylene bags, which contained 20 L of water and a 20 L oxygen headspace.

Within 1/2 hour, they were transported by boat to the Jackfish Bay study site. Each bag of fish was floated in an individual exposure cage in order that the water temperature in the bag could gradually come to within 1.5^{o}C of ambient levels, prior to release of the fish into the cage. The control fish were the last batches to be released into their (2) cages.

b) Fish Cages

The cages used in this study were constructed with a 1.9 cm (3/4") polyvinylchloride (PVC) pipe frame in order to hold a 0.6 cm (1/4") mesh nylon bag. They had a triangular top and bottom, three sides each 1 m in length and a depth of 0.5 m.

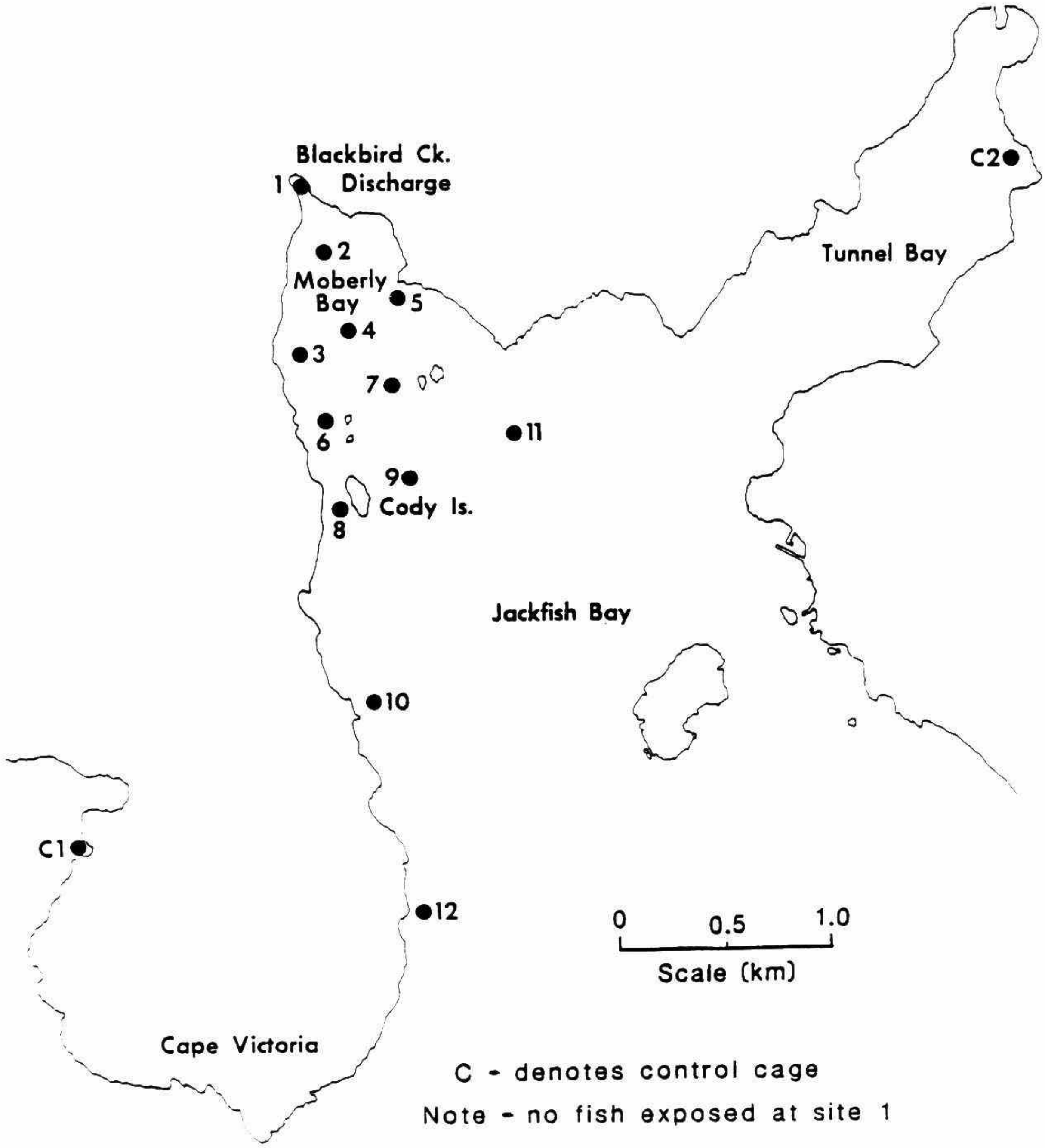
The cages were free-floating with the cage top at the water surface and were held in place by a rope and anchor. An empty plastic container was also tied to the cages as a navigational marker.

c) Cage Locations

The fish exposure grid was set-up with the aid of plume data supplied by the MOE Great Lakes Section and was concentrated in Moberly Bay with a bias toward the western shore (Figure 2). The sites were defined by triangulation using two fixed points, or by eye if sited near a distinct landmark.

Site 1 was situated at the nearest navigable point to the Blackbird Creek discharge and is considered to be representative of the creek discharge. It was the only sampling site (in Figure 2) at which a cage was not located, due to an excessive water temperature at the start of the exposure (24.5^{o}C)

FIGURE 2. FISH EXPOSURE SITES.



The two control sites, C1 and C2, were located 7.4 and 4.0 km from Blackbird Creek by water, respectively, and were considered to be beyond the influence of the plume. One site (C1) was located in a small cove on the eastern shore of Victoria Bay and the other (C2) was on the northeastern shore of Tunnel Bay. (Figure 2)

d) Procedure

The cages were sited in pre-selected locations on the day before the study commenced. On the following day (July 10th), fifty fish were placed into each cage and observations were made twice daily for fish mortality or loss of equilibrium. A fish was considered dead if after mild prodding, there was no visible respiration or other movement. Dead fish were removed immediately upon observation and discarded.

After the 96-hour exposure, five sets of composite samples containing 5 whole fish each were selected from individual sites for analysis of chlorophenols. Those fish were sacrificed, individually weighed and measured, wrapped in hexane-washed foil and frozen for preservation. A pre-exposure set of fish composites were also sampled at the Thunder Bay lab for chlorophenol analyses, the day before starting the exposure in Jackfish Bay.

The study dates, study days and number of hours exposed at a point during the study are expressed as:

Date	Time	Study Day	Exposure Time
July 10	1330 h	1	0 hours
July 12	1330 h	3	48 hours
July 14	1330 h	5	96 hours

3. Physical and Chemical Analyses.

a) List of Analyses.

i) Industrial effluent indicators - BOD, COD, TSS, TDS.
ii) General water quality tests-alkalinity, hardness, pH, conductivity.
iii) Metals - Cu, Pb, Zn, Cd, Cr, Al, Fe, Hg.
iv) Organics - total reactive phenolics, chlorophenols, resin and fatty acids.
v) Specific contaminants - NH_3, NO_2, sulfides.
vi) Nutrients - phosphorus, total soluble phosphorus, Na, K, Mg, Ca, Cl, SO_4, TKN.
vii) On-site analyses - temperature, conductivity, pH and dissolved oxygen.

b) Water Sampling Program

i) Plant Intake and Effluent

Grab samples were collected daily during the 5-day study for all parameters listed in 3a. A 24-hour composite sample of the effluent was also collected commencing at the time of the day 1 grab sample and lasting until the day 2 grab sample collection. It was analyzed for the same parameters as the grab samples with the exception of Cr, Hg, NH_3 and sulfides.

ii) Blackbird Creek discharge (Site 1).

All parameters in 3a) were analyzed on daily grab samples from this site.

iii) Jackfish Bay Field Sites

Daily grab samples were collected during the 5-day study and analysed for all parameters listed in 3a except the chlorophenols, resin and fatty acids. The chlorophenol samples were collected on day 3 and 5 of the study while the resin and fatty acid samples were collected on day 1 only. Sampling occurred at approximately the same time each day.

c. The Calculation of Effluent Concentrations for the Field Study

Effluent concentrations for the field sites, expressed as percentages, are calculated using the relative conductivity relationship between the specific cage sites and the Blackbird discharge. Relative conductivity (CR) is defined as:

$$CR = \frac{Cs - Ca}{Cb - Ca}, \quad \text{where}$$

Cs = conductivity at a particular cage site
Cb = conductivity of the Blackbird Creek discharge
Ca = ambient conductivity level at the control site

4. Laboratory Bioassays

Aerated 96-hour bioassays of the Kimberly Clark influent, effluent and Blackbird Creek discharge (site 1) were performed at the MOE/EPS mobile facility in Thunder Bay, using 1-3g rainbow trout fingerlings. In each bioassay, 10 fish were exposed per test solution and sample concentrations ranged from 10 to 100%. All dilutions were prepared using dechlorinated and carbon-filtered Thunder Bay tap water.

During the initial 12-hour period of the test, fish mortality observations were undertaken at 1/2, 1,2,4,8 and 12 hours; and at least once daily thereafter at 24, 48, 72 and 96 hours. All test solutions were kept within a temperature range of $15^{o}C \pm 2^{o}C$ and dissolved oxygen levels of the test solutions were maintained above 7.0 mg/L for the duration of the tests.

For certain cases in which an effluent's pH was less than 5.0, concurrent tests were undertaken on split samples. One set of solutions was pH-adjusted and the other was left unadjusted. The adjustment was on an individual solution basis whereby pH's were raised from below 5.0 to a range of 6.5-7.1 using a 10% solution of sodium hydroxide.

Acute lethality results were presented as 96-hour LC50s (concentrations of the samples lethal to 50% of the fish after a 96-hour exposure), along with their 95% (or greater) confidence limits; and as LT50's (time to 50% lethality) in the undiluted mill effluent. Lethality data for the 65% test solutions was compiled in order to determine whether the sample met the federal requirement of at least 80% fish survival in 65% effluent after a 96-hour exposure. For situations in which all of the fish died in the 45 and 100% test solutions and no 65% concentration was tested, it was assumed that the sample would have failed the federal bioassay test requirement.

All test requirements and procedures met the MOE fish bioassay protocol (Craig et al, 1983).

RESULTS

1. Acute Lethality

a) Field Exposure

All of the test fish died within 1.5 hours at site 2, which was located 0.3 km from the Blackbird Creek discharge. No additional mortalities were observed at any of the other sites during the initial 6 hours of the exposure. Observations and the collection of chemical samples on the second day of the study were not possible due to a dense fog which prevented boat travel to the study area.

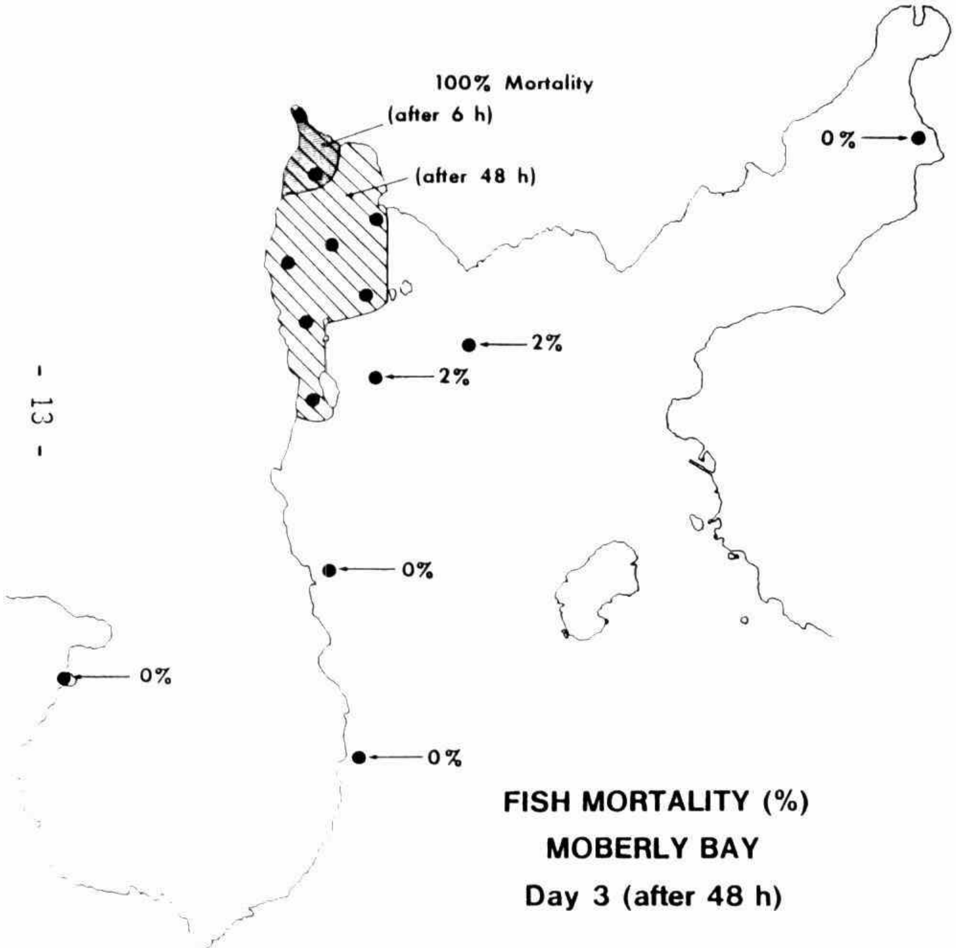
Within 48 hours, all of the test fish had died in Moberly Bay (sites 2-7); and at site 8, located between Cody Island and the western shore of Jackfish Bay, 1.5 km from the Blackbird Creek discharge (Figure 3). No further significant lethality was observed at the remaining sites in Jackfish Bay for the balance of the study (Figure 4).

b) Laboratory Bioassays

All samples of the Kimberly Clark final effluent and Blackbird Creek discharge were acutely lethal to the test fish (Tables 1 and 2).

All of the final effluent samples failed the federal regulatory test requirement for pulp and paper wastes of at least 80% fish survival in a 65% effluent concentration (Env. Can., 1971). The most lethal sample had an LC50 of 8% (Day 3 sample, Table 1). A pH adjustment of the test solutions from <5.0 to between 6.5 and 6.7 reduced lethality in that sample to an LC50 of 12%.

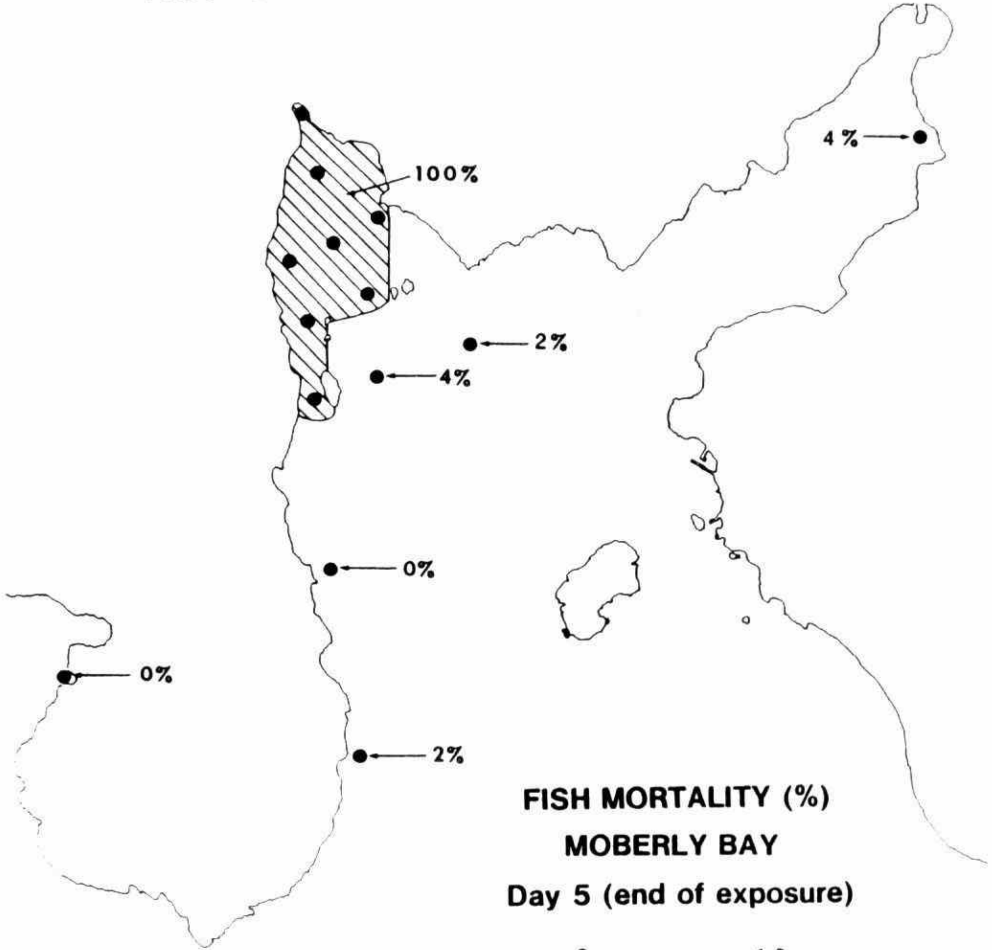
FIGURE 3.



FISH MORTALITY (%)
MOBERLY BAY
Day 3 (after 48 h)

0 0.5 1.0
Scale (km)

FIGURE 4.



FISH MORTALITY (%)
MOBERLY BAY
Day 5 (end of exposure)

0 0.5 1.0
Scale (km)

Table 1: Laboratory bioassay results for the Kimberly Clark final outfall.

Sample Type	Sample Day	Bioassay[1] LC50 (%v/v)[3]	Fish Mortality/Test Solution: Effluent Concentration (% v/v)	Fish Mortality/Test Solution: Fish Mortality (%)	LT50[2] for 100% v/v Effluent (h)
			100	100	4
grab	1	16	30	100	
		(18,13)[4]	20	70	
			10	0	
			100	100	<0.5
24-h composite	1-2	14	20	100	
		(20,10)	10	0	
			100	100	<0.5
grab	2	8	20	100	
		(9,6)	10	90	
			5	0	
			100	100	>12
24-h composite	2-3	22	30	100	<24
		(24,19)	20	10	
			10	0	
			100	100	2
grab	4	17	30	100	
		(20,13)	20	60	
			10	0	
			100	100	>12
grab	5	41	65	60	<24
		(52,32)	40	60	
			20	0	

LC50 $\bar{x}$ = 18%

S.D. = 12%

range = 8-41%

N = 6

1 Results calculated using the Moving Average Method or Binomial Test Method

2 LT50-time required to produce 50% fish lethality in the 100% effluent

3 % v/v represents: volume of effluent/volume of dilution water, expressed as a percentage.

4 Upper and lower 95% confidence levels, respectively.

Table 2: Laboratory bioassay results for the Blackbird Creek discharge to Moberly Bay. (collected at site 1; Fig. 2)

Sample[1] Day	Bioassay[2] LC50 (%v/v)	Fish Mortality/Test Solution: Effluent Concentration (%v/v)	Fish Mortality/Test Solution: Fish Mortality (%)
		100	50
1	100	45	0
		30	0
		100	100
3	37	45	100
	(45,30)[3]	30	0
		100	100
4	44	45	90
	(49,38)	30	0
		100	100
5	73	65	0
	(>100,50)	45	30[4]
		30	0

LC50 $\bar{x}$ = 58%

S.D = 34%

range = 37-100%

N = 4

[1] All were grab samples

[2] Results calculated using the Moving Average Method or Binomial Test Method

[3] Upper and lower 95% confidence limits, respectively.

[4] If considered as anomolous mortality and ignored, the LC50 would have been 81%.

LT50 results (time to 50% fish lethality) for the full strength final effluent occurred within 24 hour in each of the bioassays. Furthermore, in the 24-hour composite sample of the final effluent, collected from day 1 to day 2, as well as the day 2 grab sample, lethality was virtually instantaneous with an LT50 of less than 1/2 hour. The LT50 was not calculated for the pH adjusted sample of day 2.

In regard to the Blackbird Creek discharge, two of four samples passed the federal toxicity test requirement, with the least toxic sample having produced 50% lethality in the full strength effluent concentration (day 1 sample, Table 2).

2. Chlorophenol Bioconcentration

Total reactive phenolics were measured in water samples from Jackfish Bay (Figure 5) and in the Kimberly Clark final effluent at levels as high as 900 and 2000 ug/L, respectively.

Chlorophenol species detected in the Kimberly Clark final effluent were:

i) 2,4,5 Trichlorophenol

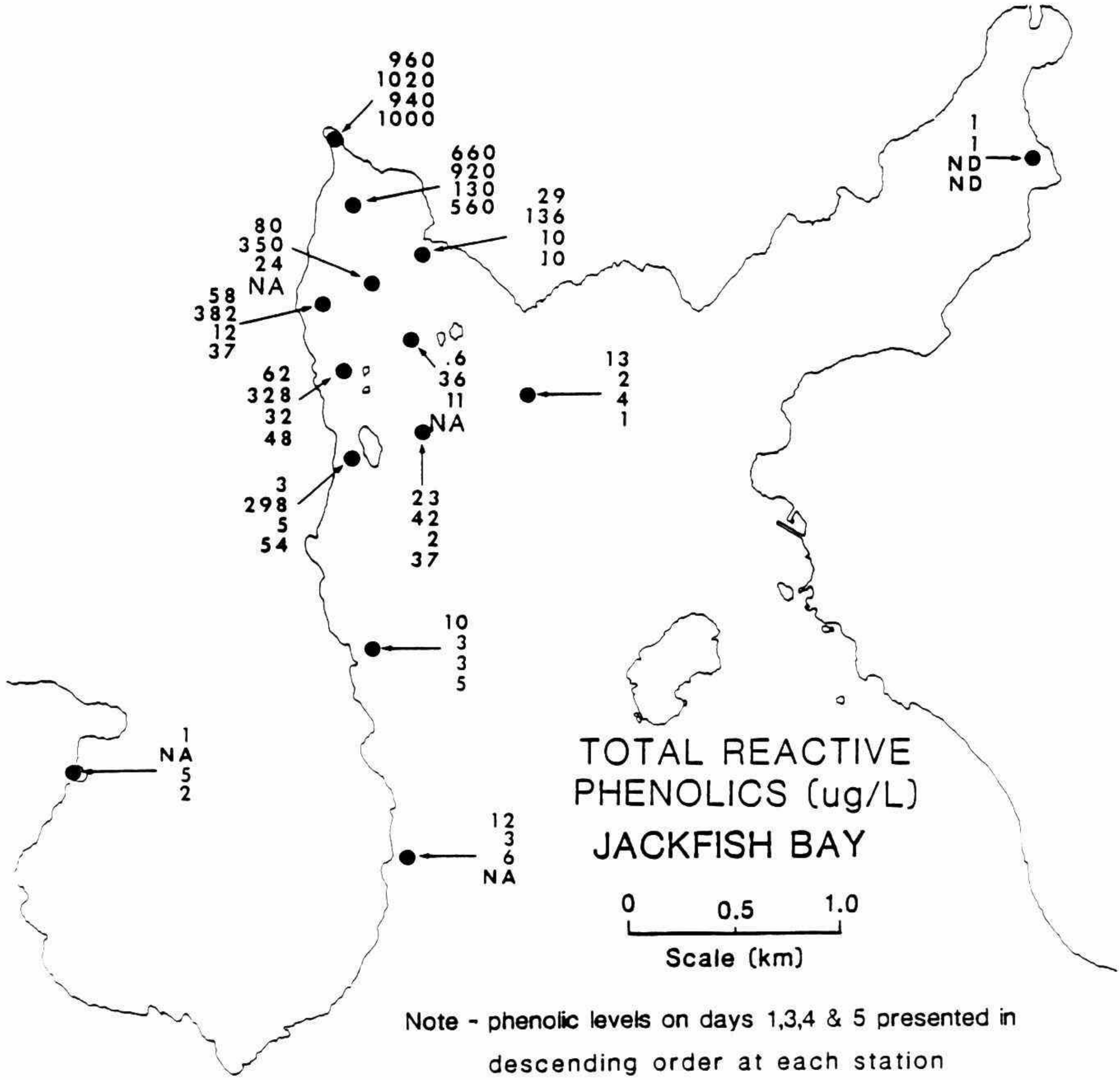
ii) 2,4,6 Trichlorophenol

iii) 2,3,4,5 Tetrachlorophenol

and iv) Pentachlorophenol

Pentachlorophenol and 2,4,6-trichlorophenol were the most prevalent chlorophenols in the mill effluent over 5 days, with mean concentrations of 11,650$\pm$ 3462 and 1260$\pm$ 671 ng/L, respectively (Table 3). They were also the sole chlorophenol species found in Jackfish Bay samples on study days 3 and 5 (Figures 6 and 7, respectively).

FIGURE 5.



TOTAL REACTIVE PHENOLICS (ug/L)
JACKFISH BAY

0 0.5 1.0
Scale (km)

Note - phenolic levels on days 1,3,4 & 5 presented in descending order at each station

N A - not available

N D - not detectable

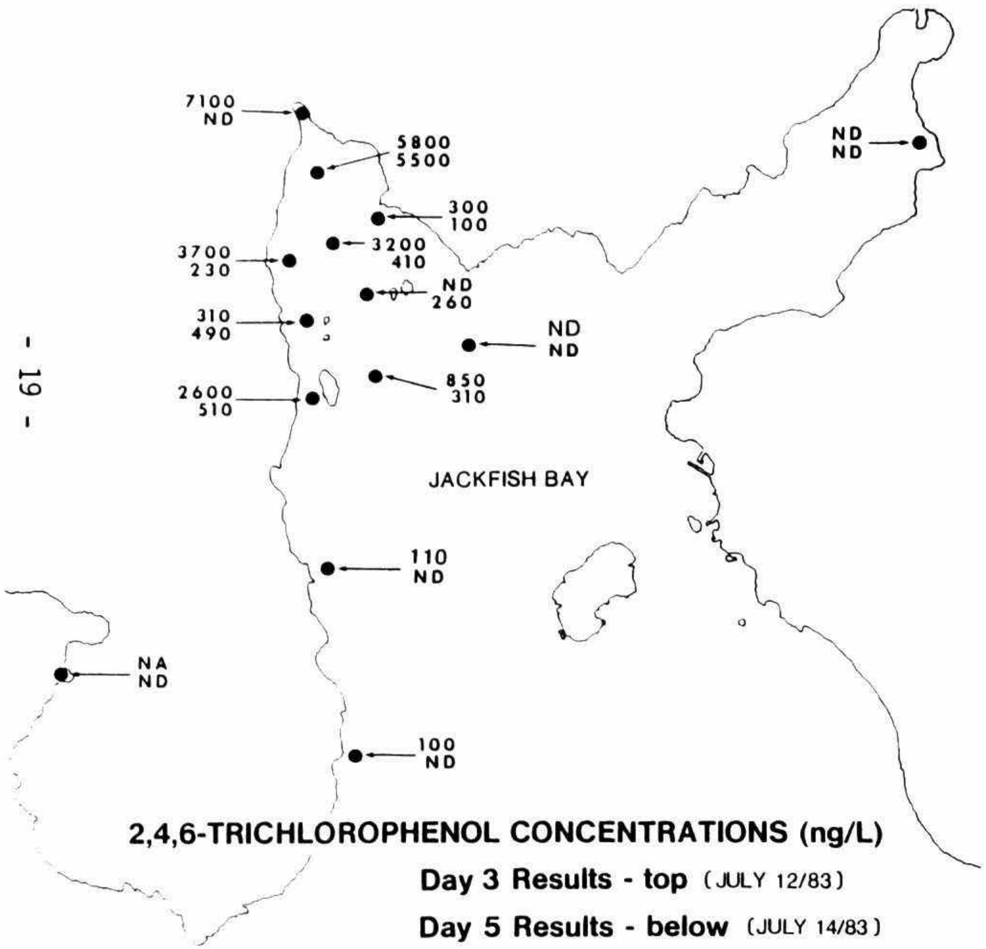
Table 3: Chlorophenol concentrations measured in the Kimberly Clark, Terrace Bay, mill effluent (July 10-14, 1983).

Chlorophenol Species	Chlorophenol Concentration (ng/L) on Exposure Day					
	1	2	3	4*	5	$\bar{x} \pm SD$
2,3,4 Trichlorophenol	ND	ND	ND	-	ND	-
2,4,5 Trichlorophenol	50	ND	ND	-	ND	-
2,4,6 Trichlorophenol	11600	16200	7800	-	11000	11650 $\pm$ 3472
2,3,4,5 Tetrachlorophenol	ND	200	ND	-	ND	-
2,3,5,6 Tetrachlorophenol	ND	ND	ND	-	ND	-
Pentachlorophenol	870	2250	820	-	1100	1260 $\pm$ 671

*N.B. - Day 4 samples lost in transit to the lab.

Note: The retention time in the analysis of 2,3,4,6-tetrachlorophenol is the same as 2,3,5,6; so that the results for the 2,3,5,6 species also includes levels of the 2,3,4,6 species present in the sample.

FIGURE 6.



2,4,6-TRICHLOROPHENOL CONCENTRATIONS (ng/L)

Day 3 Results - top (JULY 12/83)

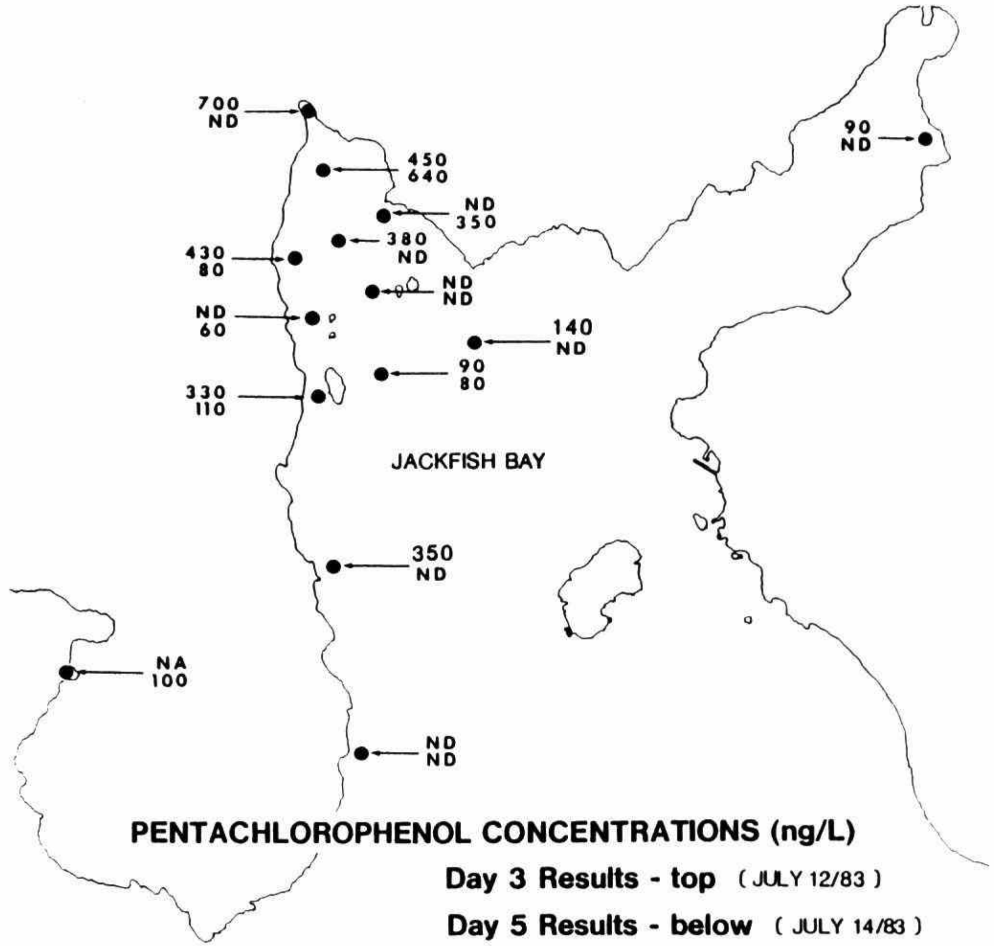
Day 5 Results - below (JULY 14/83)

0 0.5 1.0
Scale (km)

ND- not detectable

NA- not available

FIGURE 7.



PENTACHLOROPHENOL CONCENTRATIONS (ng/L)

Day 3 Results - top (JULY 12/83)

Day 5 Results - below (JULY 14/83)

0 0.5 1.0
Scale (km)

ND- not detectable

NA- not available

Detectable levels of 2,4,6-trichlorophenol were measured in subsamples of fish from three cage sites (Table 4). The highest average level occurred in fish samples from site 9, located to the east of Cody Island and approximately 1.5 km from the Blackbird Creek discharge (Figure 2). There was also a trace amount of 2,4,6-trichlorophenol in a pre-exposure control sample, however no chlorophenols were detected in fish from either control site. Pentachlorophenol residues were not found in any of the fish samples that were analyzed (detection level, >50 ng/g).

DISCUSSION

1. Acute Lethality

a) Field Exposure

The acute effects of the Kimberly Clark effluent plume on fish caged in Jackfish Bay appeared to be due to a combination of effluent-related constituents and physical characteristics.

i) Resin Acids

Leach and Thackore (1977) found that the major toxic constituents in effluents from Canadian softwood kraft pulping operations were seven resin acids: palustric, neoabietic, sandaracopimaric, abietic, dehydroabietic, isopimaric and pimaric. The latter four constituents were present at site 2, with concentrations estimated to be 1/2, 1/4, 1/5 and 1/6th of their respective LC50's for rainbow trout, as reported by Tomlinson (1981). These levels, in combination with a pH of 6.5, could account for the lethality observed at site 2 over a 96-hour period, if their individual toxic contributions were additive. However, such an effect within 1.5 hours, solely due to resin acid levels is not likely. It would have required at least a 14-fold greater level of any one of those resin acids in their more toxic sodium salt form (soaps), in order to produce an LT50 in 1.5 hours (based on work by Leach and Thakore, 1973).

Table 4: Chlorophenol concentrations measured in fish which were caged in Jackfish Bay, July 10-14, 1983

Cage Site	2,4,6 Trichlorophenol Concentrations (ng/g) in each of three composite samples		
	1	2	3
9	118	176	ND
11	70	55	ND
12	71	ND	ND
control (C1)	ND	ND	ND
Pre-exposure fish	61	ND	ND

NOTE: i) site 10 fish samples destroyed in a laboratory accident.

ii) 2,3,4 and 2,4,5 trichlorophenols; 2,3,5,6-; 2,3,4,6-; 2,3,4,5 tetrachlorophenols and pentachlorophenol not detected in any of the fish samples.

iii) detection level > 50 ng/g except > 100 ng/g for 2,3,4 trichlorophenol.

iv) The mean ($\bar{x}$) and standard deviation (S.D.) for the site 9 samples would be 106 $\pm$ 76, if the 3rd composite sample (ND) from that site had a value of 1/2 the detection level. This variability of results between samples is not unlike that encountered by Suns (pers. com.). In 3 sets of samples of yearling yellow perch caught at sites in the Great Lakes during 1981 and 82, the S.D. for 2,4,6 trichlorophenol levels for each set of samples was equivalent to between 50 and 100% of their respective mean values.

Furthermore, the laboratory bioassay of Blackbird Creek on day 1 provided additional evidence that resin acid levels, in themselves, could not account for the fish lethality at site 2. In other words, although resin acid levels in the laboratory bioassay were 40-60% greater than those at site 2, the sample was substantially less lethal with only 10% fish mortality in the full strength solution after 24 hours. The pH levels did not appear to have played a role in the differences for the lab sample pH was 6.0 as compared with a value of 6.5 in the field at site 2.

Therefore resin acids appear to be only one of several potential contributors to fish lethality on day 1. No resin or fatty acid analysis of day 3 samples were possible due to lab limitations.

ii) Temperature

The Blackbird Creek discharge at site 1 (the closest access to the creek by boat) had mid-day water temperatures which ranged from 22-25^{o}C over the 5-day study. These temperatures were similar to levels which would be expected for some of the northshore rivers and streams. For example, both the Black and White Rivers do exceed a temperature of 20^{o}C in the summer (MOE, 1979). Furthermore, the 15 km distance of Blackbird Creek from mill to lake and a 3-5 day effluent residence time in the creek (Draper pers. com.) would have allowed for the dissipation of a considerable amount of waste heat prior to the final discharge.

During the study, water temperatures in Moberly Bay dropped an average of 5.5^{o}C between sites 1 and 2, with a more gradual decline out into the bay. (Appendix II). The background water temperatures for nearshore Lake Superior were 11-13^{o}C.

Regardless of their source, elevated temperatures close to the creek discharge would likely have increased the toxic effects of the bleached kraft mill effluent (BKME) to fish. Loch and MacLeod (1974) found that salmonid mortality in a BKME test solution at 19^{o}C, was double that of an identical sample at 11.5^{o}C over a 120-hour exposure. The mode of action appeared to involve an increase in fish metabolism; resulting in an increased respiration rate, toxicant contact with the gills per unit time, and hence in a greater toxicant uptake rate.

The temperature of 20.5^{o}C, recorded at site 2 during the observation of complete fish mortality after a 1.5-hour exposure, is considered to be within the range of tolerance for rainbow trout. Black (1953) found that rainbow trout would survive at a temperature of 22.4^{o}C, when acclimated to 11^{o}C water. The acclimation temperature for fish in this study was between 11 and 14.5^{o}C.

Test site temperatures of 12-17^{o}C on day 3 were not a major factor in test fish mortality. As a consequence, mortality during the study does not appear to have been a direct result of exposure temperature at the cage sites.

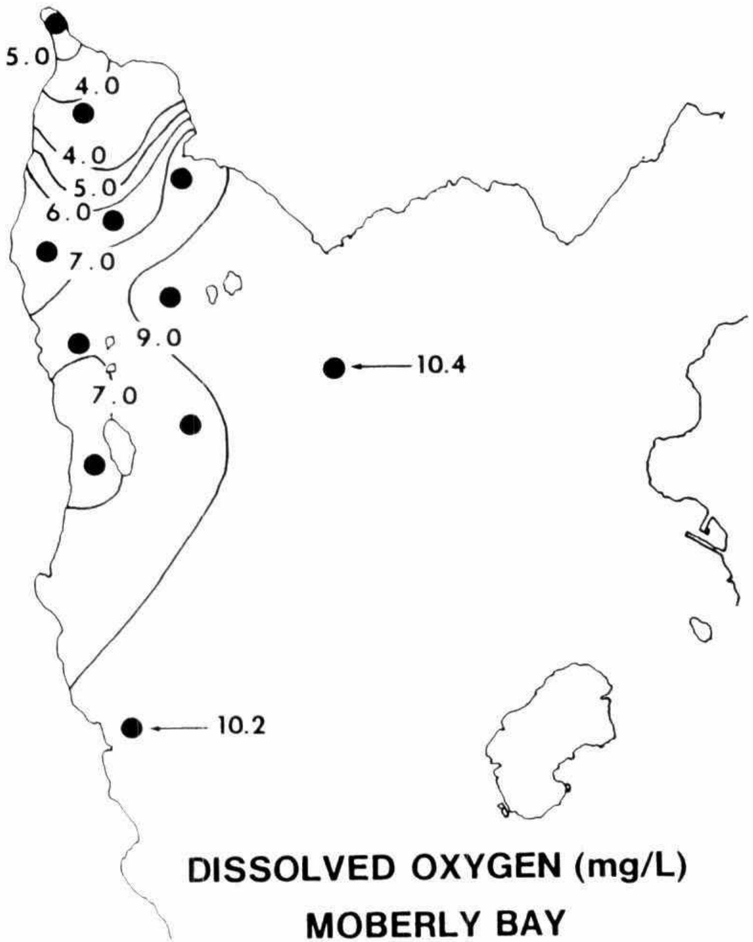
iii) Dissolved Oxygen

Several authors have shown that a decrease in dissolved oxygen concentrations will increase the toxicity of poisons to fish (Lloyd, 1961). Hicks and De Witt (1971) reported an inverse relationship between dissolved oxygen (D.O.) and the toxicity of kraft mill effluents, while Brouze (1975) noted that any BOD discharge which might reduce D.O. below 7-8 mg/L will begin to stress the fish, making them more susceptible to natural environmental pressures. The U.S. Dept. of the Interior (1967) recommended a minimum dissolved oxygen of 5 mg/L for the protection of fish, while Doudoroff and Shumway (1970) recommended a minimum of 5 mg/L in warm water and 6-7 mg/L in cold water.

An oxygen level of 3.8 mg/L was measured at site 2 during the 1.5-hour period in which complete fish mortality occurred (Figure 8). That level does not meet the MOE Dissolved Oxygen Objective, which states that, "at no time should D.O. be less than 5 mg/L for cold water biota" at $20^{o}C$ (MOE, 1978). No lethal effects were observed at any of the other sites during the first 6 hours of the exposure and corresponding oxygen levels at those sites were greater than or equal to 6.5 mg/L. (Appendix III)

The 48-hour lethality observed in Moberly Bay (Figure 3) was likely due to a combination of reduced oxygen levels in the plume and the presence of toxic compounds from the mill effluent. Dissolved oxygen levels measured at 48 hours for sites 3-8 inclusive were 2.6, 3.4, 7.8, 3.2, 8.6 and 2.8 mg/L, respectively (Figure 9). With but two exceptions (7.8 and 8.6 mg/L), these values were well below the MOE objective of 5 mg/L for the protection of aquatic life (MOE, 1978); and were so low that they, in themselves, could have caused fish lethality.

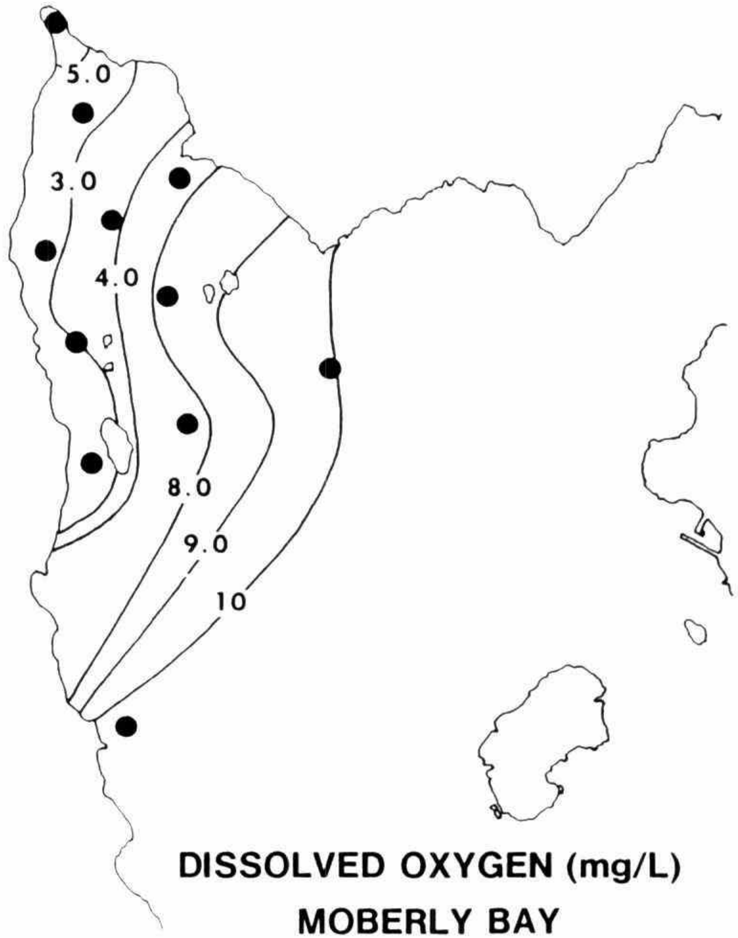
FIGURE 8.



DISSOLVED OXYGEN (mg/L)
MOBERLY BAY
Day 1

0 0.5 1.0
Scale (km)

FIGURE 9.



DISSOLVED OXYGEN (mg/L)
MOBERLY BAY
Day 3

0 0.5 1.0
Scale (km)

However, the lethality observed at 48 hours was most likely due to a combination of reduced oxygen levels in the plume and toxic components from the mill effluent.

Dissolved oxygen levels at the exposure sites varied greatly from day to day. For example, 5 stations had D.O. levels of less than 4.0 mg/L on day 3, while on day 4, only one station had a level of less than 7.7 mg/L. The oxygen levels on day 5 were the second lowest of the study with the levels at sites 1, 2, 3, 4, 6 and 8 unable to meet the criteria for cold water fish. In all cases in which D.O. was below 5.0 mg/L, oxygen saturation at the prevailing water temperature was not the limiting factor. Furthermore, the day to day fluctuations in D.O. appeared to be due to the prevailing wind and wave action.

Based on the results of this study, dissolved oxygen was a very important factor in fish mortality in Moberly Bay.

b) Laboratory Bioassays

i) A Comparison of Results for the Kimberly Clark Final Effluent and Blackbird Creek Discharge

The final effluent was approximately twice as lethal as the creek discharge, with LC50 values ranging from 8-41% for the effluent and 37-100% for the creek samples (Table 1 and 2). However, there was no significant statistical difference in mean LC50 values for the final mill effluent and Blackbird Creek discharge due to the variability in lethality during the study ($p>.05$; t-test). No comparison of results for the two discharges was made on individual days, due to the time of effluent travel from the mill to the Blackbird Creek outfall of 3-5 days.

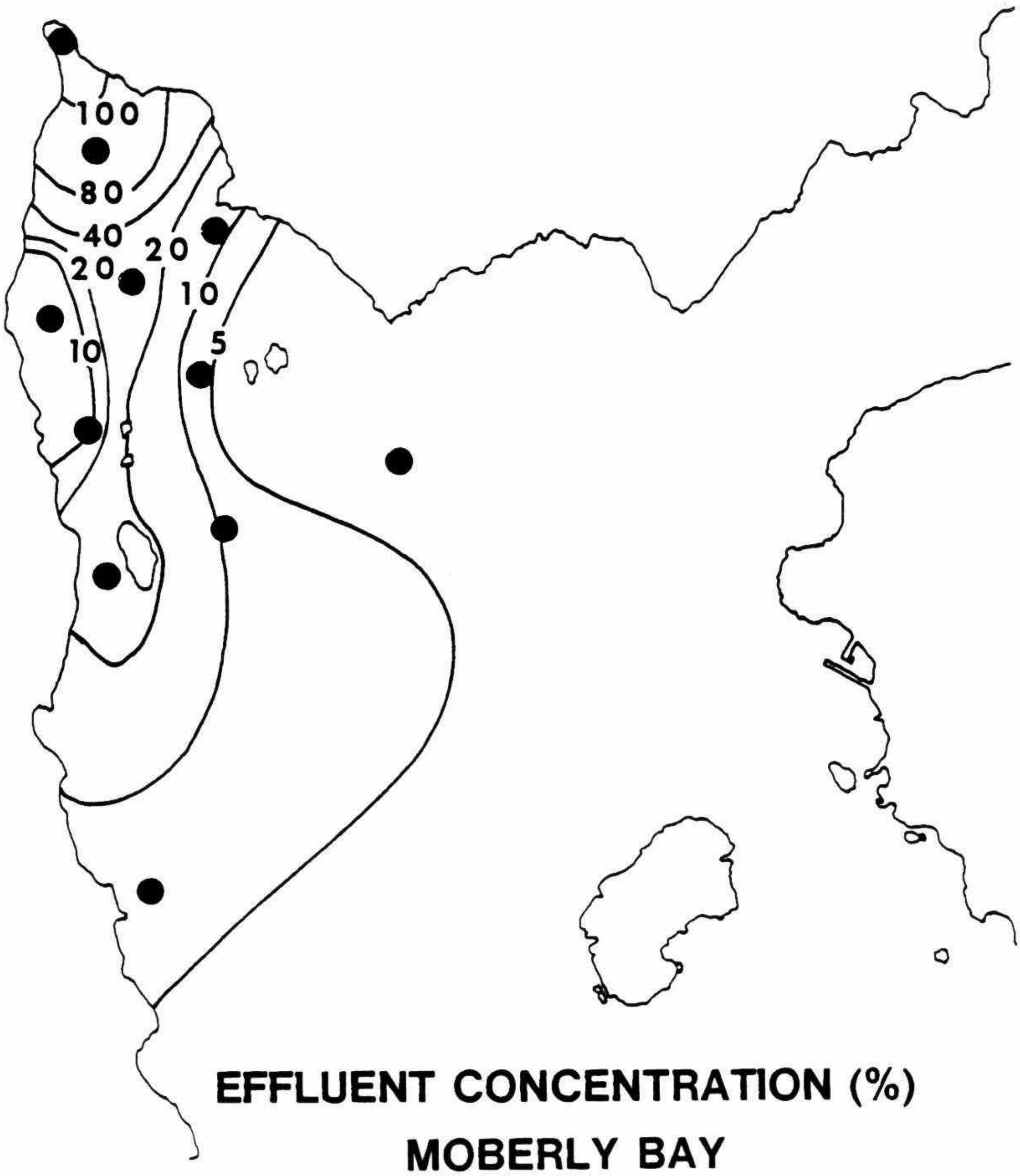
There was also a discernible difference in the test results of the two effluents as they relate to the federal regulatory bioassay requirement (Env. Can., 1971). The Blackbird Creek discharge samples passed the federal test in two of four cases, while the final effluent failed to meet the requirements in each of four grab samples and two 24-hour composites.

Although the creek discharge samples were collected at the closest access to the discharge (site 1) rather than the creek mouth itself, it would appear that some detoxification and/or dilution of the effluent occurred during the 15 km creek flow the from mill to Lake Superior. No stream flow measurements were taken during this study; however, Holloran (1984) calculated that the natural watershed contribution to streamflow averaged 23,000 cu m/d (5 MGD) during July 1981. This value represents 13% of the estimated effluent flow from the mill at the time of sampling in this study. During the full year natural stream flows were quite variable with monthly averages representing between 2% (January) and 61% (April) of total flow (Holloran, 1984).

ii) A Comparison of Laboratory and Field Bioassay Results

A laboratory bioassay of the Blackbird Creek (site 1) sample collected on day 1 produced 50% fish mortality within 72 hours. On the other hand, the field bioassay at site 2, which was slightly more dilute than the creek effluent (Figure 10), produced complete fish mortality in 1.5 hours. Therefore, it appears that the lab bioassay provided an underestimation of the effects observed in the field on the first day of the study.

FIGURE 10.



EFFLUENT CONCENTRATION (%)
MOBERLY BAY
Day 1

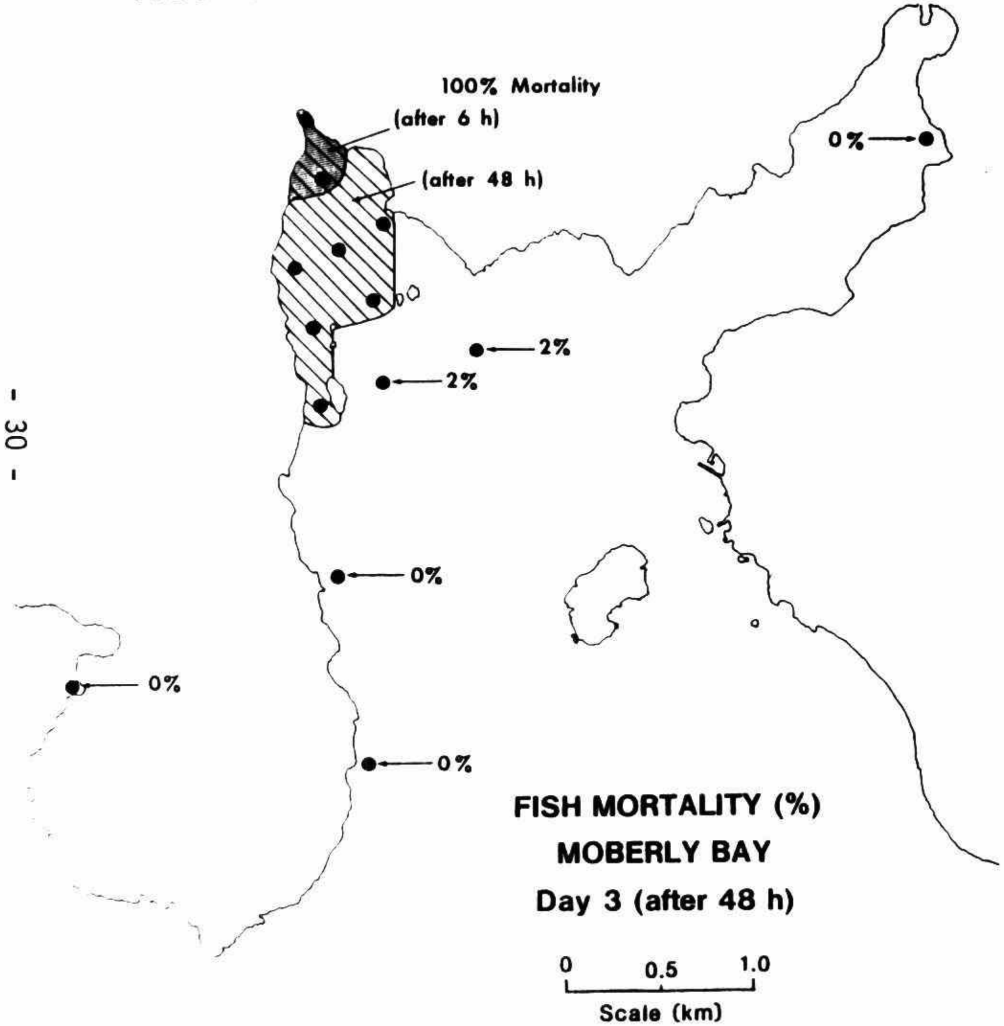
0 0.5 1.0
Scale (km)

The difference in results between the lab and field tests on day 1 may have been due to the differences in dissolved oxygen levels under which each test was undertaken. The oxygen level in the field was 3.8 mg/L at the end of the fish exposure while the bioassay test solutions in the lab were maintained at levels of greater than 7.0 mg/L.

The laboratory bioassay sample collected from Blackbird Creek on day 3 was lethal with 100% mortality in an effluent dilution of 45%. During the collection of that sample, all of the test fish were found dead at field sites 3-8 inclusive. Based on relative conductivity levels at the field sites, the estimated effluent concentration at 4 of those 6 sites which were lethal, ranged from 40-46% (Figures 3 and 11, p. 30). These values are consistent with the laboratory results which demonstrated complete mortality in a 45% laboratory test solution within 48 hours. Therefore, the lab bioassay was a good indicator of field results for at least two-thirds of the lethal observations in the field, and may have been even better had observations and conductivity measurements been possible on day 2 of the study.

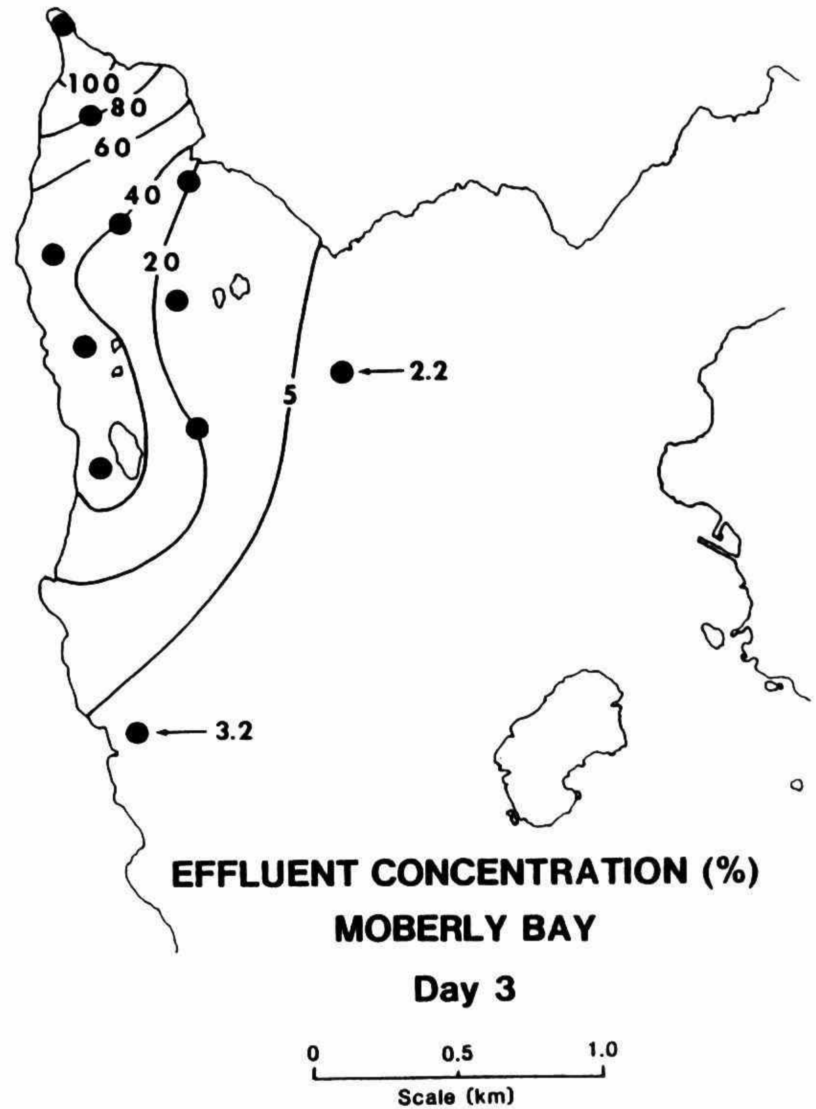
Although effluent concentrations at a majority of the lethal field sites on day 3 were similar to the lab bioassay result, dissolved oxygen levels were much different. Concentrations of less than 4.0 mg/L were recorded in the field and greater than 8.0 mg/L in the 45% bioassay solution. Therefore, the variables and the degree to which each contributed to lethality may have been different in each case, but the ultimate results were comparable.

FIGURE 3.



FISH MORTALITY (%)
MOBERLY BAY
Day 3 (after 48 h)

FIGURE 11.



EFFLUENT CONCENTRATION (%)
MOBERLY BAY
Day 3

iii) A Comparison of 1981 and 1983 Laboratory Bioassay Data

The LC50 results for the final effluent in 1981 ranged from 6-17% for 24-hour composite samples over a consecutive 8-day period; while an 8-41% range was recorded for a combination of grab and composite samples in this study (Table 2). The current results showed an improvement over those from 1981 in that the median LC50 result in the latter case was between 9-11% (there is no single median value in an even number of samples, i.e. 8) versus 16-17% (N = 6) for 1983. A comparison of the Blackbird Creek discharge samples was not undertaken due to differences in sampling locations.

3. Chlorophenol Bioconcentration

After the collection and analyses of water samples from Jackfish Bay, the MOE Laboratory discovered pentachlorophenol in some bottle cap liners which were similar to the ones used in this study. The level of contamination, if present, was in the order of 50-100 ng/L. All pentachlorophenol values presented in figure 7 are as reported by the laboratory, and in some samples the values are in excess of the potential level of contamination by a factor of from 3-7 times, clearly indicating that the Kimberly Clark wastewater discharge was contributing pentachlorophenol to Jackfish Bay.

The species of chlorophenols which were found in Moberly Bay (2,4,6-tri-and pentachlorophenol) were also found to be consistent with those present at the highest concentrations in the mill effluent of Kimberly Clark (Figure 6 and 7, Table 3). The levels of 2,4,6 tri-and pentachlorophenol discharged from Blackbird Creek on day 3 of the study were 60 and 55% of the mean ($\bar{x}$) concentration measured in the final effluent over 5 days. A 2,4,6-trichlorophenol concentration of 7100 ng/L at site 1 on day 3, represented the highest chlorophenol concentration measured in Jackfish Bay while detectable levels were found as far as 3.5 Km down the western shore of Jackfish Bay. The area of detection for pentachlorophenol was limited to Moberly Bay proper, and levels discharged from Blackbird Creek were approximately 1/10th of those for trichlorophenol.

The concentrations of the two chlorophenol compounds found in Jackfish Bay were consistent with levels reported by Cherwinsky (1983). However, Cherwinsky reported levels of 2,3,5,6 tetrachlorophenol ranging from 60-600 ng/L in Jackfish Bay, whereas this compound was not detected in the final effluent, creek discharge or Moberly Bay during this study.

Test fish, which survived the 96-hour field exposure, bioaccumulated detectable levels of chlorophenols in their flesh (Table 4). At two sites (11,12), trichlorophenol levels in fish were measured at or above the detection level of 50 ng/g (ppb) while the highest levels were found at site 9, located 1.5 km from the Blackbird Creek discharge. In those samples, 2,4,6-trichlorophenol was detected in two of three cases with levels of 118, 176 and <50 ng/g, while water samples at that site registered 850 and 310 ng/L on days 3 and 5, respectively. These results illustrate that waterborne 2,4,6 trichlorophenol in the high ppt range (ng/L) can be bioconcentrated by rainbow trout in the low ppb range (ng/g) over a 5-day period. No comparable studies were available in the literature for trichlorophenol uptake by rainbow trout, however, similar results were reported by Nimii and McFadden (1982) for pentachlorophenol. They found uptake in the range of 39-119 ng/g in rainbow trout exposed to a 660 ng/L level of sodium pentachlorophenate over a 20-day period.

The maximum bioconcentration factor (BCF) for 2,4,6 trichlorophenol in a composite sample of fish from site 9 was found to be 370; based on the relationship:

$$BCF = \frac{fc - fb}{Wr}, \text{ where}$$

fc = highest 2,4,6 trichlorophenol level measured in fish from site 9 after 96-h exposure (176 ng/g)

fb = the background level of 2,4,6 trichlorophenol found in a composite of pre-exposure fish (61 ng/g)

Wr = 2,4,6 trichlorophenol level in water at site 9 on day 5 (310 ng/L for the day on which fish were sampled).

A BCF for 2,4,6 trichlorophenol of 370 within 5 days is significant, but not unexpected. Freitag et al (1982) calculated a BCF for 2,4,6 trichlorophenol of 310 using a European cyprinid, the orfe (Leuciscus idus), which was exposed to a trichlorophenol concentration of 30 ug/L over a 3-day period.

Stern and Walker (1978) indicated concern for BCF's of greater than 100, by stating that at such levels a number of questions must by raised; one of which involves the chronic effects of the levels found in fish. The most significant effect of chlorophenol bioconcentration in this study may indirectly involve fish lethality. An NRCC report (1982) states that chlorophenols cause an acceleration of oxygen consumption by fish. In our study, such an effect in combination with depressed oxygen levels at the cage sites may have added further stress and ultimately contributed to the lethal results.

The area of the lake in which elevated chlorophenol concentrations could result in their uptake by fish was limited to Moberly Bay and the western shore of Jackfish Bay and therefore the potential for chlorophenol bioconcentration would appear to be fairly localized. Confirmation of this conclusion was demonstrated by young of the year spottail (Notropis hudsonius) shiners that were caught in Tunnel Bay (Figure 2) during September, 1983. They contained no detectable levels of chlorophenols when analyzed as whole fish composite samples (Suns, pers, com.).

CONCLUSIONS

1. The Kimberly Clark mill effluent was not in compliance with the fish survival requirement of the federal government in regard to the regulation of pulp and paper mill wastes.

2. The mill effluent remained acutely lethal to fish at the Blackbird Creek discharge, 15 km downstream of the mill input, in each of 4 daily grab samples. In 2 of these 4 cases, the discharge samples did not meet the federal requirement for fish survival in pulp and paper mill effluents.

3. The pulp mill effluent produced fish lethality in the surface waters of Moberly Bay and along the western shore of Jackfish Bay to Cody Island in an *in situ* bioassay.

4. The plume from Blackbird Creek caused dissolved oxygen in portions of Jackfish Bay to drop below the MOE objective at certain times during the study.

5. Depressed dissolved oxygen levels were considered an important factor in this study, and likely acted in combination with toxic constituents, such as resin acids, to produce the fish lethality observed in the field.

6. The laboratory and field results for fish lethality were quite similar, and where they didn't agree, the lab results were an underestimation of those observed in the field.

7. A short-term (96-hour) field assay using rainbow trout was a useful tool to define the extent of an acutely lethal discharge to the natural environment and to identify the potential for contaminants uptake by fish exposed in a pulp mill plume.

8. A significant uptake of chlorophenols by fish occurred at a maximum distance of 1.5 km from the Blackbird Creek discharge, and therefore, would appear to be a localized effect only.

RECOMMENDATIONS

Regarding:

(A) The Kimberly Clark Mill Effluent

1. Kimberly Clark of Canada Ltd. should fulfill its control order requirements which include a reduction in both effluent toxicity and the levels of oxygen-demanding components of the waste.

2. The diffusion of the mill effluent in Jackfish Bay should be improved to minimize its zone of influence due to potentially depressed dissolved oxygen levels.

3. A follow-up field study should be undertaken to assess improvements in the Kimberly Clark effluent quality after conditions of the control order have been met in 1987.

(B) Future Studies of Pulp and Paper Mill Impacts on Surface Waters

1. More laboratory and field lethality comparisons are required in order to better interpret laboratory results.

2. Future field studies of pulp mill wastes should include aerated and unaerated laboratory bioassays of water samples from cage sites in order to better assess the effects of dissolved oxygen levels on fish lethality.

3. A program should be undertaken to quantitatively identify pulp and paper mill effluent contaminants present in indigenous biota near pulp mill discharges. The contaminant bioavailablity results could be related to currently available contaminant inventories of each pulp mill under consideration and to literature assessments of the potential sub-lethal effects of those pulp mill contaminants to aquatic biota.

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APPENDICES

Appendix I: Resin Acid Concentrations in the Kimberly Clark Mill Influent, Effluent and at the Fish Exposure Sites in Jackfish Bay, July 10-14, 1983.

	Resin Acid Concentrations (ug/L)							
DATE SAMPLED (STUDY DAY)/ SAMPLE SITE	PIMARIC	SANDARACO-PIMARIC	LEVO-PIMARIC	ISO-PIMARIC	ABIETIC	NEO ABIETIC	DEHYDRO-ABIETIC	PALUSTRIC
July 10, 1983(1)								
Plant Influent	5	ND	ND	1	ND	ND	2	ND
Plant Effluent	231	133	<10	274	805	<10	550	<10
Site 1	202	95	90	70	808	<10	596	19
2	129	67	70	142	556	<10	430	<10
3	9	1	ND	9	4	3	9	ND
4	19	7	ND	14	5	1	13	ND
5	12	3	ND	5	ND	1	9	ND
6	21	10	42	14	ND	2	11	ND

Appendix I: Resin Acid Concentrations of the Kimberly Clark Mill Influent, Effluent and at the Fish Exposure Sites in Jackfish Bay, July 10-14, 1983.

Resin Acid Concentrations (ug/L)

DATE SAMPLED (STUDY DAY)/ SAMPLE SITE	PIMARIC	SANDARACO-PIMARIC	LEVO-PIMARIC	ISO-PIMARIC	ABIETIC	NEO ABIETIC	DEHYDRO-ABIETIC	PALUSTRIC
July 10, 1983(1) Continued								
Site 7	ND	ND	ND	ND	ND	ND	ND	ND
8	9	ND	2	2	1	ND	2	ND
9	11	2	ND	6	ND	ND	<5	ND
10	11	1	ND	ND	ND	1	2	ND
11	21	8	31	14	ND	1	15	ND
12	7	ND	ND	1	1	1	1	ND
C1	4	ND	ND	ND	ND	ND	ND	ND
C2	3	ND	ND	ND	ND	ND	ND	ND

Appendix I: Resin Acid Concentrations of the Kimberly Clark Mill Influent, Effluent and at the Fish Exposure Sites in Jackfish Bay, July 10-14, 1983.

Resin Acid Concentrations (ug/L)

DATE SAMPLED (STUDY DAY)/ SAMPLE SITE	PIMARIC	SANDARACO-PIMARIC	LEVO-PIMARIC	ISO-PIMARIC	ABIETIC	NEO ABIETIC	DEHYDRO-ABIETIC	PALUSTRIC
July 10, 1983(1)								
Plant Influent	5	5	ND	ND	ND	2	1	<1
Plant Effluent	197	275	125	365	1300	<10	523	38
July 12, 1983(3)								
Plant Influent	6	ND	ND	1	ND	1	1	ND
Plant Effluent	120	60	305	102	404	<10	236	38
July 13, 1983(4)								
Plant Influent	5	ND	ND	ND	ND	ND	4	ND
Plant Effluent	170	133	560	194	756	<10	333	42
Site 1	198	91	<10	177	790	<10	447	<10
July 14, 1983(5)								
Plant Influent	ND	ND	ND	ND	ND	ND	ND	ND
Plant Effluent	292	106	227	384	278	<10	760	58
Site 1	178	82	264	164	482	<10	392	39

Appendix II: Surface Water Temperatures at the Fish Exposure Sites in Jackfish Bay, July 10-14, 1983

Sample Date (Day)/Time	Surface Water Temperature (°C) at the Fish Exposure Sites[1]						
	1	2	3	4	5	6	7
July 10 (1) 1440 h	24.5	20.5	15.0	15.0	15.0	14.5	13.5
1930 h	25.0	24.0	16.0	16.0	17.0	15.0	16.0
July 12 (3) 0800 h	22.0	19.5	17.0	16.0	14.0	16.5	14.0
2000 h	24.0	12.0	12.0	10.5	12.0	12.0	11.0
July 13 (4) 0830 h -	22.0	13.5	11.5	12.0	12.0	12.0	11.5
July 14 (5) 0800 h	23.0	18.5	13.0	12.5	13.0	13.0	12.5

Sample Date (Day)/Time	8	9	10	11	12	C1	C2
July 10 (1) 1440 h	16.0	14.0	13.0	14.0	12.0	12.0	15.0
1930 h	15.5	15.0	12.8	15.0	12.0	11.5	16.5
July 12 (3) 0800 h	16.0	14.0	13.0	13.0	12.0	-	14.5
2000 h	13.0	12.0	12.0	13.8	12.5	13.0	16.5
July 13 (4) 0830 h -	12.0	12.0	12.0	11.5	11.0	11.0	14.5
July 14 (5) 0800 h	13.0	13.0	12.0	13.0	11.0	11.5	15.0

1 exception - site 1 had no fish exposed at it.

NB - no observations or chemical samples possible on Day 2 (July 11/83) due to bad weather.

Appendix III: Surface Water Dissolved Oxygen Levels at the Fish Exposure Sites in Jackfish Bay, July 10-14, 1983

Sample Date (Day) Time	Dissolved Oxygen Levels (mg/L) at the Fish Exposure Sites[1]						
	1	2	3	4	5	6	7
July 10 (1)	5.2	3.8	7.0	6.5	8.6	7.2	10.0
July 12 (3)	5.2	2.8	2.6	3.4	7.8	3.2	8.6
July 13 (4)	4.3	7.8	8.4	8.7	8.8	8.2	9.1
July 14 (5)	4.2	3.5	4.8	4.6	10.1	4.3	5.0
	8	9	10	11	12	C1	C2
July 10 (1)	6.5	9.0	9.3	9.8	10.4	11.6	10.4
July 12 (3)	2.8	6.8	10.4	10.2	11.0	-	10.0
July 13 (4)	10.2	9.5	9.5	9.8	10.4	10.0	
July 14 (5)	4.6	9.5	10.3	10.2	11.4	10.4	10.0

1 exception - site 1 had no fish exposed at it.

NB - no observations or chemical samples possible on Day 2 (July 11/83) due to bad weather.